



School of Medicine
Department of Physiology
East Carolina University
Brody Medical Sciences Building • Greenville, NC 27858-4354
252-816-2770 office • 252-816-3460 fax

August 6, 1999

Office of Naval Research
800 North Quincy Street
Arlington, VA 22217-5660

Dear Sir or Madam:

I am writing to inform you that I have accepted an Assistant Professor position at East Carolina University School of Medicine effective June 1, 1999.

The project # N00014-96-1-0563, entitled "Expression and Function of Heat Shock Proteins in the Mammalian Gut during Experimentally Induced Hypoxia and Exogenous Stress" has been completed. Please see enclosed Final Technical report.

Sincerely yours,

A handwritten signature in black ink, appearing to read "Alexander K. Murashov".

Alexander K. Murashov, M.D., Ph.D.
Assistant Professor in Physiology
East Carolina University School of Medicine
Brody Bldg, #6N-61
600 Moye Blvd,
Greenville, NC 27858
Phone: 252-816-3111
Fax: 252-816-3460
Email: amurashov@brody.med.ecu.edu

19990813 033

REPORT DOCUMENTATION PAGE

*Form Approved
OMB No. 0704-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Service, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188) Washington, DC 20503.

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

| | | | | | | | | |
|---|--|--|---|--|------------------------------|--|---|--|
| 1. REPORT DATE (DD-MM-YYYY) | | | 2. REPORT DATE | | 3. DATES COVERED (From - To) | | | |
| 08.05.1999 | | | Final | | 03/01/1996-06/01/1999 | | | |
| 4. TITLE AND SUBTITLE | | | 5a. CONTRACT NUMBER | | | | | |
| EXPRESSION AND FUNCTION OF HEAT SHOCK PROTEINS IN THE MAMMALIAN GUT DURING EXPERIMENTALLY INDUCED HYPOXIA AND EXOGENOUS STRESS | | | 5b. GRANT NUMBER N00014-96-1-0563 | | | | | |
| 6. AUTHOR(S) | | | 5c. PROGRAM ELEMENT NUMBER 96PR04375-00 | | | | | |
| Murashov, Alexander K. | | | 5d. PROJECT NUMBER | | | | | |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) | | | 5e. TASK NUMBER | | | | | |
| Columbia University 630 West 168th Street, New York, NY 10032 | | | 5f. WORK UNIT NUMBER | | | | | |
| 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) | | | 8. PERFORMING ORGANIZATION REPORT NUMBER | | | | | |
| Office of Naval Research ONR 252 800 North Quincy Street Arlington, VA 22217-5660 | | | 10. SPONSOR/MONITOR'S ACRONYM(S) | | | | | |
| 12. DISTRIBUTION AVAILABILITY STATEMENT | | | 11. SPONSORING/MONITORING AGENCY REPORT NUMBER | | | | | |
| APPROVED FOR PUBLIC RELEASE | | | | | | | | |
| 13. SUPPLEMENTARY NOTES | | | | | | | | |
| 14. ABSTRACT <p>The research was focused on characterization of the role of heat shock proteins during exogenous stress using mouse as model. The results of experiments provided evidence that heat shock protein 25 (Hsp25) may be a specific factor protecting neuromuscular system from stress and stimulating subsequent regeneration. Hsp25 was specifically expressed in lower cholinergic motorneurons in normal conditions and was rapidly induced after heat shock and hypoxia. Hsp25 was colocalized with p38 and AKT kinases in the same cells. Specific p38 kinase inhibitor blocked expression of Hsp25 during regeneration. p38/AKT/Hsp25 cascade of signaling is critical for cell recovery.</p> | | | | | | | | |
| 15. SUBJECT TERMS <p>Heat shock proteins, exogenous stress, hypoxia, regeneration, gut, neuromuscular system, Map kinases, AKT kinase, Hsp25</p> | | | | | | | | |
| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | | 18. NUMBER OF PAGES | | 19a. NAME OF RESPONSIBLE PERSON Alexander Murashov | |
| a. REPORT UU | | | b. ABSTRACT UU | | c. THIS PAGE UU | | 19b. TELEPHONE NUMBER (Include area code) 252-816-3111 | |

FINAL TECHNICAL REPORT

GRANT #: N00014-96-1-0563

PRINCIPAL INVESTIGATOR: Dr. Alexander K. Murashov (e-mail: am96@columbia.edu), Co-Investigator, Prof. Debra J. Wolgemuth (e-mail:djw3@columbia.edu)

INSTITUTION: Columbia University

GRANT TITLE: Expression and Function of Heat Shock Proteins in the Mammalian Gut During Experimentally Induced Hypoxia and Exogenous Stress

REPORTING PERIOD: 1 March 1996 - 1 June 1999

AWARD PERIOD: 1 March 1996 - 1 June 1999

OBJECTIVE: To characterize the role of the heat shock proteins (hsp) in the cellular response to hypoxic cell damage in the gut; to determine the function of the hsp in the cellular adaptation to hypoxic conditions and development of tolerance to subsequent hypoxic assaults.

APPROACH:

Hypoxia: Mice were subjected to hypoxia in a special chamber which provided a controlled atmosphere containing 6% oxygen and the balance made up of nitrogen for 2, 8, and 16 hours.
Hyperthermia: Mice were anesthetized with sodium pentobarbitol (80 mg/kg body weight) and placed on a temperature-controlled heating pad (50°C). Body temperature was raised to 42°C and maintained for 15 minutes and the animals were allowed to recover for 2, 8 and 16 hours, followed by euthanasia with CO₂.

Immunohistochemistry: After specified periods of time, control and stressed animals were euthanized with CO₂ and perfused first with saline and then with 4% paraformaldehyde in PBS (pH 7.4). Tissues were postfixed in 4% paraformaldehyde, and then processed as paraffin or frozen sections.

Antibodies: Antibodies to hsps were purchased from StressGen Biotechnologies Corp. (Victoria, Canada). Immunostaining was performed using the Elite ABC Kit according to manufacturer's instruction (Vector Laboratories Inc., Burlingame, CA). Controls for specificity included: tissues processed without incubation in primary antibody, secondary antibody, or ABC complex solutions.

ACCOMPLISHMENTS: We have examined the spatio-temporal pattern of expression of members of the hsp cellular stress gene family at the protein level in the gut of adult mice subjected to experimental hypoxia and heat shock. Immunohistochemical detection in histological sections showed induction of the Hsp70 inducible family member in the mouse stomach after 2, 8 and 16 hours of hypoxia. The expression was observed in Chief cells, which secrete pepsinogen, and in Parietal cells, which secrete hydrochloric acid. Expression of Hsp32 (heat shock protein encoding Heme Oxygenase-1) was detected in the stomach at 8 hours after heat shock, in Parietal cells and in surface mucous cells of gastric pits. Expression of Hsp25 was induced in circumferential and longitudinal layers of the smooth muscles in all regions including the stomach, duodenum, small and large intestine. In the stomach, strong induction was also detected in squamous epithelium. In small intestine, the expression was restricted to Goblet cells, which secrete mucinogen and in lamina propria of villi. In lamina propria, the expression was localized to smooth muscle cells and lymphocytes. In large intestine, the expression of Hsp25 was detected in Goblet cells and Paneth cells, which secrete lysozyme. The results indicate that hsps are expressed in different cellular populations and in different patterns after hypoxia

and heat shock. We also made the important observation that Hsp25 is specifically localized to primary motor neurons and fibers of the spinal cord and the brain stem, and is strongly induced after hypoxic injury. This indicate that Hsp25 can be a critical factor protecting function of mammalian gut at different levels including the smooth muscle and secreting cells, the fibers innervating them and motor neurons. Axotomy performed on sciatic nerve showed that Hsp25 is strongly induced in motor neurons on the 4th and subsequent days after injury and corresponds to the pattern of regeneration. The expression of Hsp25 co-localized with p38 kinase and Mapkapk-2 kinase and AKT. Injection of specific p38 kinase inhibitor blocked Hsp25 expression. Thus Hsp25 is implicated as a downstream factor of p38 Map kinase pathway which may play an important role in regeneration. Immunofluorescence on primary cell culture of sympathetic neurons showed co-localization of Hsp25 with F-actin in individual growth cones. This observation suggests that Hsp25 may be an actin binding protein. Hsp25 may promote axonal growth and regeneration through reorganization of actin filaments in nerve cells. AKT kinase a downstream member of PI-3 kinase pathway was also activated during regeneration. AKT plays an important role in protection from cell death. The pattern of expression of AKT recapitulated the expression patter of Hsp25. Immunohistochemistry showed co-localization of AKT and Hsp25 to the same cell populations during regeneration. Furthermore, immunofluorescence on primary cell culture of sympathetic neurons showed co-localization of Hsp25 and AKT in individual growth cones.

SIGNIFICANCE: An understanding of the molecular mechanisms of cellular responses to hypoxia is a first step to identifying new methods for therapy of ischemic injury. Our studies showed that the cells of the mammalian gut with high secretory functions and functional load appeared to be particularly sensitive to oxygen depletion. Upregulation of Hsp25 at different levels of the gastro-intestinal system argue that this protein can be a critical factor required for protection and subsequent recuperation. These findings indicate that hsps can serve as molecular markers of hypoxic injury in different cellular populations of the gut, as well as predict the cell and tissues most likely to be affected by ischemia. Our findings further demonstrated that Hsp25 is an important factor for regeneration. Hsp25 promotes regeneration in injured cells by regulating actin cytoskeleton and interaction with p38 kinase and AKT kinase.

PUBLICATIONS AND ABSTRACTS :

1. Murashov, A.K. and Wolgemuth, D.J. (1996) Expression of heat shock proteins in the gut after hypoxia and exogenous stress. Abstract presented 9 September 1996, Cell Biology of Hypoxia, Naval Medical Research Institute, p. 13.
2. Murashov, A.K., Talebian, S. and Wolgemuth, D.J. Role of Stress Protein Hsp25 in Cellular Response of Motor Neurons to Stress and Injury. In: *Ischemic Stroke, IBC's 6th Annual Conference, November 6-7, 1997, Washington, DC.*
3. Murashov, A.K., Ul Haq, I., Park, E. and Wolgemuth, D.J. Role of the Hsp25 and p38 kinase pathway in axonal regeneration of spinal motor neurons. In: *28th Annual Meeting Society for Neuroscience, Los Angeles, CA, November 7-12, 1998, p.1845.*
4. Murashov, A.K., Talebian, S. and Wolgemuth, D.J. Role of stress protein hsp25 in the response of orofacial nuclei motor system to physiological stress. *Mol. Brain Research*, 1998, 63, 14-24.
5. Murashov, A.K., Talebian, S., Bauer, R., and Wolgemuth, D.J. Selective expression of different members of the heat shock family in the enteroendocrine cells of the gut after experimentally induced hypoxia and heat shock (in preparation).

REPORT OF INVENTIONS AND SUBCONTRACTS

(Pursuant to "Patent Rights" Contract Clause) (See Instructions on back)

The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services Directorate for Information Operations and Reports (9000-0085), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not conform with a collection of information that is valid OMB control number.

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THIS ADDRESS. RETURN COMPLETED FORM TO THE CONTRACTING OFFICER.

1.a. NAME OF CONTRACTOR/SUBCONTRACTOR c. CONTRACT NUMBER 2.a. NAME OF GOVERNMENT/PRIME CONTRACTOR c. CONTRACT NUMBER

| | | |
|--|---|---|
| c. CONTRACT NUMBER N00014-96-1-0563 | d. AWARD DATE (YYYY/MM/DD) 19960301 | b. ADDRESS (Include Zip Code) 630 West 168th Street New York, NY, 10032 |
|--|---|---|

SECTION I - SUBJECT INVENTIONS

6. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR/SUBCONTRACTOR (None, "so state") none

| | | | |
|--|--------------------------|--|---|
| a. NAME(S) OF INVENTOR(S) (Last, First, Middle Initial) | b. TITLE OF INVENTION(S) | c. DISCLOSURE NUMBER, PATENT APPLICATION SERIAL NUMBER OR PATENT NUMBER | d. ELECTION TO FILE PATENT APPLICATION (X) |
| Murashov, Alexander K. | NONE | N/A | X |

| | | |
|---|---|--|
| e. EMPLOYER OF INVENTOR(S) NOT EMPLOYED BY CONTRACTOR/SUBCONTRACTOR | f. EMPLOYER OF INVENTOR(S) NOT EMPLOYED BY CONTRACTOR/SUBCONTRACTOR | g. ELECTED FOREIGN COUNTRIES IN WHICH A PATENT APPLICATION WILL BE FILED |
| (1) (a) NAME OF INVENTOR (Last, First, Middle Initial) | (2) (a) NAME OF INVENTOR (Last, First, Middle Initial) | (1) TITLE OF INVENTION |
| (b) NAME OF EMPLOYER | (b) NAME OF EMPLOYER | (2) FOREIGN COUNTRIES OF PATENT APPLICATION |
| (c) ADDRESS OF EMPLOYER (Include Zip Code) | (c) ADDRESS OF EMPLOYER (Include Zip Code) | |

SECTION II - SUBCONTRACTS (Containing a "Patent Rights" clause)

6. SUBCONTRACTS AWARDED BY CONTRACTOR/SUBCONTRACTOR (None, "so state") none

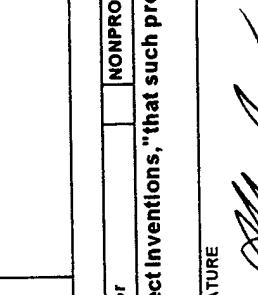
| NAME OF SUBCONTRACTOR(S) | ADDRESS (Include Zip Code) | SUBCONTRACT NUMBER(S) | FAR "PATENT RIGHTS" | | SUBCONTRACT DATES (YYYY/MM/DD) |
|--------------------------|----------------------------|-----------------------|----------------------------|-----------------------|--------------------------------|
| | | | (1) CLAUSE d. NUMBER | (2) DATE (YYYY/MM) | |
| None | b. | | | | |

7. CERTIFICATION OF REPORT BY CONTRACTOR/SUBCONTRACTOR (if required if (X as appropriate))

I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.

a. NAME OF AUTHORIZED CONTRACTOR/SUBCONTRACTOR
OFFICIAL (Last, First, Middle Initial)
Murashov Alexander

b. TITLE
Associate Research Scientist

c. SIGNATURE


d. DATE SIGNED
08/05/1999

PREVIOUS EDITION MAY BE USED.

NONPROFIT ORGANIZATION

SMALL BUSINESS

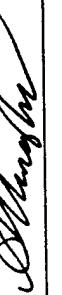
SECTION III - CERTIFICATION

7. CERTIFICATION OF REPORT BY CONTRACTOR/SUBCONTRACTOR (if required if (X as appropriate))

I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.

a. NAME OF AUTHORIZED CONTRACTOR/SUBCONTRACTOR
OFFICIAL (Last, First, Middle Initial)
Murashov Alexander

b. TITLE
Associate Research Scientist

c. SIGNATURE


d. DATE SIGNED
08/05/1999

WHSDIOR, Jan 99